

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Mello et al.

Serial No.:

09/490,291

Group No.:

1653

Filed:

01/20/99

Examiner:

H. Schnizer

Entitled:

Novel Purification And Fiber Spinning Techniques For Protein Fibers

TRANSMITTAL OF FORMAL DRAWINGS

Official Draftsperson BOX ISSUE FEE Assistant Commissioner for Patents Washington, D.C. 20231

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In response to the NOTICE OF INFORMAL DRAWINGS or ALLOWABILITY, attached please find:

X 12 sheets of formal drawing(s) for this application.

🗵 Each sheet of drawing indicates the identifying indicia suggested in 37 CFR

§ 1.84(c) on the front side of the drawing.

Respectfully submitted:

Dated: APNIL 29, 2003

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Reg. No. 29,579

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Group Art Unit: 1653

Applicant: Mello et al.

Novel Purification And Fiber

Spinning Techniques For Protein

Fibers

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ATGAGAGGATCGCATCACCATCACGGATCCATGGCTAGCGGTAGAGGCGGGCTGGGTGGCCAG GGTGCAGGTGCGGCTGCCGCGGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGC GCGGCAGCGGCCGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGT AFAGGCGGGCTGGGTGCAGGTGCGGCTGCGGCTCCCGCGGCAGCGGCCGCAGGCGGTGC CGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGG TGCAGGTGCGGCTGCCGCGCGCGCAGCGGCCGCAGGCGGTGCCGAAGGTGGCTATGGCGGCCT GGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCGC GGCAGCGGCCGGCGGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAG AGGCGGGCTGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGCCAGG CGGCCTGGGTTCTCAGGGGACTAGTGGGATCCGTCGACCTGCAGCCAAGCTTAATTAG

MRGSHHHHHHGSMASGRGGLGGQGAGAAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAA AAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLG GQGAGAAAAAAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAAAAAAAGGAGQGGYGGLGSQ GGYGGLGSOGTSGIRRPAAKLN

FIG. 2

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ATGAGAGGATCGCATCACCCATCACGGATCCATGGCTAGCGGTAGAGGCGGGCTGGGTGGCCAG GGTGCAGGTGCGGCTGCCGCGGCAGCGGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGC GCGGCAGCGGCGCGGGGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGT AGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCGCGGCAGCGGCCGCAGGCGGTGC CGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGG TGCAGGTGCGGCTGCCGCGCGCGCAGCGGCCGCAGGCGGTGCCGAAGGTGGCTATGGCGGCCT GGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAACTAGCGGTAGAGGCGGGCT GGGTGGCCAGGGTGCAGGTGCGGCTGCCGCCGCAGCGGCGCAGGCGGTGCCGGCCAAGGTG GCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCAGGTGCGG CTGCGGCTGCCGCGGCAGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGG GGACTAGCGGTAGAGGCGGGCTGGGTGCCAGGGTGCAGGTGCGGCTGCCGCGGCAGCGGCC GCAGGCGGTGCCGAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTG GGTGGCCAGGGTGCAGGTGCGGCTGCCGCCGCCAGGCGGCCAAGGTGG CTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAACTAGCGG TAGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCGCGCAGCGGCGGCAGCGGTG CCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGG GTGCAGGTGCGGCTGCCGCGGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCC TGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCGGCTGCCG CGGCAGCGGCCGGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTA GAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCGGCAGCGGCAGGCGGTGCC GGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAA CAAACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCGCGGCAGCGGC CGCAGGCGGTGCCGAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCT GGGTGGCCAGGGTGCGGCTGCGGCTGCCGCGCAGCGGCGCAGGCGGTGCCGGCCAAGGTG GCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCAGGTGCGG CTGCGGCTGCCGCGGCAGCCGCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGG GGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCGCGGCAGCGGCC GCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTAT GGTCCGGGTCAACAACTAGTGGGATCCGTCGACCTGCAGCCAAGCTTAATTAG

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FIG. 4

ATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCATGGCTAGCGGTAGAGGCGGGCTGGGT GGCCAGGGTGCAGGTGCGGCTGCCGCGGCAGCGGCCGCAGGCGGTGCCGGCCAAGGTGGCTAT GGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCG GCTGCCGCGGCAGCGGCCGGCGGGGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACT AGCGGTAGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGGCCGCAGG $\tt CGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGG$ CGGCCTGGGTTCTCAGGGGGCTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAAACTAGCGGTAGAGG CGGGCTGGGTGCCAGGTGCGGCTGCGGCTGCCGCGGCAGCCGCCAGGCGGTGCCGGCCA AGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGG TGCGGCTGCGGCTGCCGCCAGCGCCGCCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTC TCAGGGGACTAGCGGTAGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCCGCGGCAG CGGCCGCAGGCGGTGCCGAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGCG GGCTGGGTGGCCAGGTGCGGCTGCCGGCTGCCGGCAGCCGCAGGCGGTGCCGGCCAA GGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTCCGGGCGGTTATGGTCCGGGTCAACAAACT AGCGGTAGAGGCGGGCTGGCCAGGGTGCAGGTGCGGCTGCGGCTGCCGCGGCAGCGCCGCAGG CGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGCTGGGTGG CCAGGGTGCAGGTGCGGCTGCCGCGCGCAGCGGCGGCGGCGGCCAAGGTGGCTATGG CGGTAGAGGCGGGCTGGGTGCCAGGGTGCAGGTGCGGCTGCCGCGGCAGCGGCCGCAGGCG GTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCGGTTATGGTCCGG GTCAACAAACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGGTGCAGGTGCGGCTGCCGCGCCA GCGGCCGCAGGCGTGCCGAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTAGAGGC GGGCTGGGTGCCAGGTGCGGCTGCGGCTGCCGCGCAGCGGCCGCAGGCGGTGCCGGCCA AGGTGGCTATGGCGGCCTGGGTTCTCAGGGGGACTAGCGGTAGAGGCGGGGCTGGGTGGCCAGGGTGCAGG TGCGGCTGCGGCTGCCGCCAGCGCCGCCAGGCGGTGCCGGCCAAGGTGGCTATGGCGGCCTGGGTTC TCAGGGGACTAGCGGTAGAGGCGGGCTGGGTGGCCAGGTGCGGTGCGGCTGCCGCGGCAG CGGCCGCAGGCGGTGCCGAAGGTGGCTATGGCGGCCTGGGTTCTCAGGGGACTAGCGGTCCGGGCG GTTATGGTCCGGGTCAACAACTAGTGGGATCCGAATTCGAGCTCCGTCGACAAGCTTCGAGCACCACC ACCACCACCACTGA

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FIG. 6

ATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGATCCGAATTCGTGGATATGGAGGTCTTGGTGGA CAAGGTGCCGGACAAGGAGCTGGTGCAGCCGCCGCAGCAGCAGCTGGTGGTGCCGGACAAGGAGGATA TGGAGGTCTTGGAAGCCAAGGTGCTGGACGAGGTGGACAAGGTGCAGCCGCAGCCGCAGCTG GAGGTGCTGGTCAAGGAGGATACGGAGGTCTTGGAAGCCAAGGTGCTGGACGAGGAGGATTAGGTGGA CAAGGTGCAGGTGCAGCAGCAGCTGGAGGTGTCGGACAAGGAGGACTAGGTGGACAAGGTGCTGG ACAAGGAGCTGGAGCAGCTGCTGGTGGTGCCGGACAAGGAGGATATGGAGGTCTCGGAA GCCAAGGTGCAGGACGAGGTGGATCAGGTGGACAAGGGGGCAGCAGCAGCAGCAGCAGCTGGAGGT GCCGGACAAGGAGATATGGAGGTCTTGGAAGCCAAGGTGCAGGACGAGGTGGATTAGGTGGACAGGG TGCAGGTGCAGCAGCAGCAGCCGGAGGTGCTGGACAAGGAGGATACGGTGGTCTTGGTGGAC AAGGTGCCGGACAAGGTGGCTATGGAGGACTTGGAAGCCAAGGTGCTGGACGAGGAGGATTAGGTGGA CAAGGTGCAGCAGCAGCAGCTGGAGGTGCCGGACAAGGAGGACTAGGTGGACAAGGAGCTGG AGCAGCCGCTGCAGCAGCTGGTGCCGGACAAGGAGGATATGGAGGTCTTGGAAGCCAAGGTGCTG GACGAGGTGGACAAGGTGCAGCCGCAGCAGCAGCCGGAGGTGCTGGACAAGGAGGATACGGT GGACAAGGTGCCGGACAAGGAGGCTATGGAGGACTTGGAAGCCAAGGTGCTGGACGAGGAGGATTAGG TGGACAAGGTGCAGGTGCAGCAGCAGCAGCAGCTGCAGGTGCCGGACAAGGAGGATTAGGTG GACAAGGTGCAGGTGCAGCAGCAGCAGCTGGAGGTGCTGGACAAGGAGGATTAGGTGGACAAGGT GCTGGACAAGGAGCTGGAGCAGCCGCTGCAGCAGCAGCTGGTGGTGTTAGACAAGGAGG CCGGAGGTGCTGGACAAGGAGATATGGTGGTCTTGGTGGACAAGGTGTTGGACGAGGTGGATTAGGTG GACAAGGTGCAGCGCAGCGGCAGCTGTTGGTGCTGGACAAGGAGGATATGGTGGTGTTCTGGGG CGTCTGCTGCCTCTGCAGCTGCATCCCGTTTGTCTTCTCCTCAAGCTAGTTCAAGAGTTTCATCAGCTGTT TCCAACTTGGTTGCAAGTGGTCCTACTAATTCTGCGGCCTTGTCAAGTACAATCAGTAATGTGGTTTCAC AAATAGGCGCCAGCAATCCTGGTCTTTCTGGATGTGATGTCCTCATTCAAGCTCTTCTCGAGCACCACCA CCACCACCACTGAA

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 ${\tt MASMTGGQQMGRIRIRGYGGLGGQGAGQGAGAAAAAAAGGAGQGGYGGLGSQGAGGGGGGAGAAAAA}$ AGGAGQGGYGGLGSQGAGRGGLGĞQGAĞAAAAAGGVGQGGLGĞQGAGQAGAAAAAAGĞAGQGGYG GLGSQĞAGRGGSGGĞGAGAAAAAAĞGAGQGGYGGLGSQĞAGRGĞLGGQĞGAGAAAAAAAAGGAĞQGGYG GLGGQGAGQGGYGGLGSQGAGRGGLGGQGAGAAAAAGGAGQGGLGGQGAGAAAAAAGGAGQGGYGGL GSQGĀGRGĞQGAGAAAAĀAGGAGQGGYĞGQGAGQGGYGGLĞSQGAGRGGLGGQGAGAAAAĀAAAGGA GQĞGLGGQGĀGAAAAAAGGAGQGĞLGGQGĀGQGĀGAAAAAAAĀĀAAGGVRQGGŸGGLGSQGAGRGGQ GAGAAAAAAGGAGQGGYGGLGGQGVGAGGLGGQGAGAAAVGAGQGGYGGVGSGASAASAAASRLSS PQASSRVSSAVSNLVASGPTNSÄALSSTISNVVSQIGASNPGLSGCDVLIQALLGHHHHHH

FIG. 8

AEIYNKDGNKVDLYGKAVGLHYFSKGNGENSYGGNGDMTYARLGFKGETQINSDLTGYGQWEY NFQGNNSEGADAQTGNKTRLAFAGLKYADVGSFDYGRNYGVVYDALGYTDMLPEFGGDTÄYSD DFFVGRVGGVATYRNSNFFGLVDGLNFAVQYLGKNERDTARRSNGDGVGGSISYEYEGFGIVGAY GAADRINLQEAQPLGNGKKAEQWATGLKYDANNIYLAANYGETRNATPITNKFTNTSGFANKTQ DVLLVAQYQFDFGLRPSIAYTKSKAKDVEGIGDVDLVNYFEVGATYYFNKNMSTYVDYIINOIDS DNKLGVGSDDTVAVGIVYOFA

FIG. 9

ATGAGAGGATCGCATCACCATCACGATCCATGGCTAGCGGTGACCTGAAAAACAA AGTGGCCCAGCTGAAAAGGAAAGTTAGATCTCTGAAAGATAAAGCGGCTGAACTGAAACAAG AAGTCTCGAGACTGGAAAATGAAATCGAAGACCTGAAAGCCAAAATTGGTGACCTGAATAAC ACTAGTGGGATCCGTCGACCTGCAGCCAAGCTTAATTAG

FIG. 10

MRGSHHHHHHGSMASGDLKNKVAQLKRKVRSLKDKAAELKQEVSRLENEIEDLKAKIGDLNNTSGIRRPAA KLN

FIG. 11

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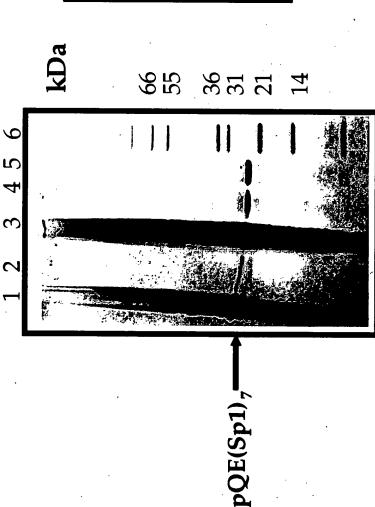
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Comparative Gel of Acid Lysis vs. Traditional Denaturing Conditions



Formic acid lysate
 Propionic acid lysate
 8M urea lysate
 6M guanidine lysate,
 Ni-NTA purified
 Formic Lysate,
 Ni-NTA purified
 Molecular weight markers

FIG. 12



Gel of QAE-Sephadex Purification of Propionic Acid (PA) ExtractedpET[(Sp1)₄/(Sp2)₁]₄Protein

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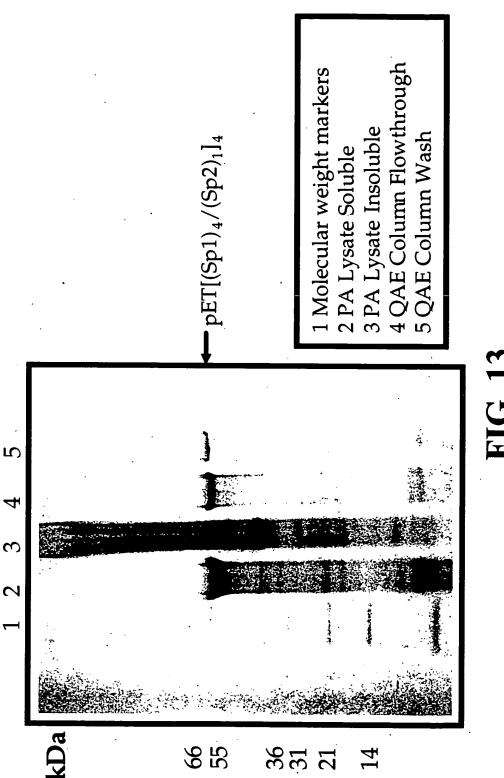
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Fibers Sheet 8 of 12 Atty. Docket No.: NA-1151 1 Propionic Acid/Gdn-HCl Lysate QAE Column Flowthrough Molecular weight markers and Guanidine-HCl Extracted pET[(SP1)₄/(SP2)₁]₄ Protein QAE Column Wash QAE-Sephadex Purification of Propionic Acid (precolumn) 0 m kDa 99 36 55 FIG. 14 3 2 pET[(SP1)₄/(SP2)₁]₄

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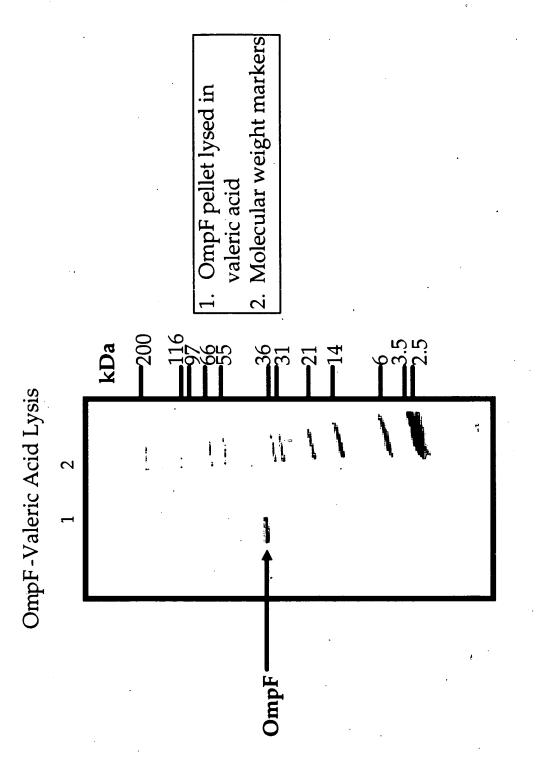


FIG. 15



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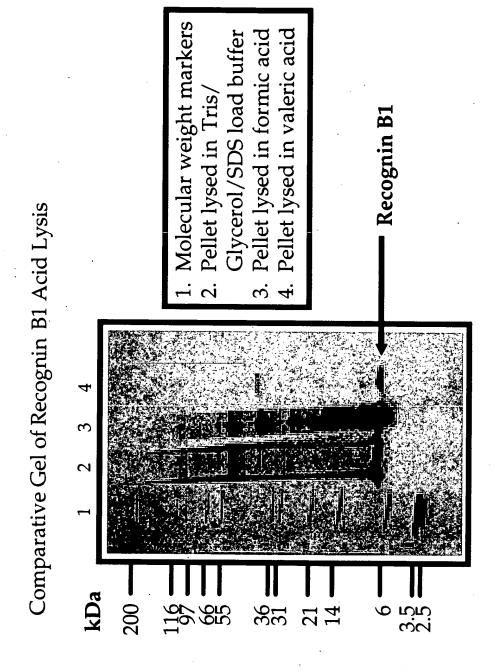


FIG. 16



pETNcDS fiber under light microscopy. Spun from 25%

protein solution into 90% methanol coagulation bath.

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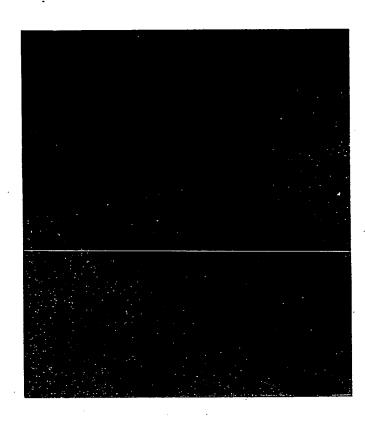
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 $pQE[(SP1)_4/(SP2)_1]_4$ fiber under light microscopy. Spun from a 12.5% protein solution into 90% methanol coagulation bath.

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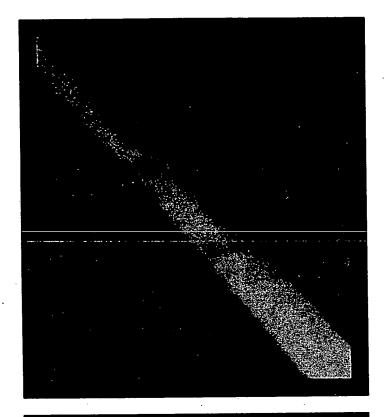
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White light

Polarized light w/ tint plate

FIG. 18